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14. ABSTRACT

In this project, we are testing the hypothesis that metastatic prostate cancer cells secrete a common signature of neuroactive peptides. We acquired two new state-of-the-art mass spectrometers and transferred and updated our existing workflows to the new instruments. Further, we established three complementary quantitation and identification MS-based proteomics workflows that allow us to interrogate the secretome of prostate cancers at unprecedented levels. We now have the most advanced setup to tackle the complex repertoire of molecular factors that are secreted by cancer cells and associated stromal cells. Ultimately, we aim to identify proteases and peptides that are responsible for tumor-induced pain and sensory neuropathy. By interfering with the generation of neuroactive peptides through specific protease inhibition, we aim to develop novel mechanism-based therapies for cancer pain.

15. SUBJECT TERMS
Proteomics, peptidomics, mass spectrometry, prostate cancer, metastasis, pain

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1. Introduction

Tumor-induced pain and sensory neuropathy are common in patients with advanced metastatic prostate cancers and significantly impact their quality of life. Cancer cells and associated stromal cells secrete a complex repertoire of factors that stimulate tumor growth and metastasis. Among them are peptides that have been shown to sensitize or directly excite primary afferent neurons. Tumor-derived peptides are a significant source of cancer pain. Such overlap between cancer growth and neuronal signaling pathways is of particular concern when cancer cells are in close proximity to neurons (e.g., in bone metastasis). Identifying neuroactive cancer-secreted peptides and understanding the roles that specific proteases play in peptide generation and clearance is a critical step in the development of novel mechanism-based therapies for cancer pain. In this project, we are testing the hypothesis that metastatic prostate cancer cells secrete a common signature of neuroactive peptides. We are using inhibitor profiling to determine which proteases are responsible for the metabolic regulation of these peptides.

2. Keywords

Proteomics, peptidomics, mass spectrometry, prostate cancer, metastasis, pain

3. Overall project summary

Dr. Hardt took a new position and moved his research team from Boston Biomedical Research Institute to The Forsyth Institute in March 2013. The grant started in August 2013. Initially, we experienced the following delays: 1) Acquisition, installation and training on the new mass spectrometry instrumentation peptides synthesis and other equipment in the laboratory: 2) hiring and training the necessary personnel. We are now fully functional in the laboratory to accomplish the proposed work. Dr. Hardt is now the faculty director of the mass spectrometry core of the newly created Forsyth Center for Salivary Diagnostics. Through this core, the research project has now access to the most advanced mass spectrometry-based technologies available today. A brief summary of the updated available instrumentation is provided in appendix Al.

The instrumentation upgrade, at the same time, meant that we needed to adjust and modulate the originally proposed analysis techniques to make them compatible with the new instrumentation. For example, a key part of Specific Aim **1** ("Define the common set of neuroactive peptides secreted by metastatic prostate cancer cell lines) is the identification of neuropeptide substrates by the PALeO method that originally was based on MALDI-MS acquired data. We expanded this analysis workflow to now include ESI-MS data that is being generated by the newly acquired state-of-the-art instruments (Thermo Scientific Orbitrap Fusion; QExactive Plus). We thoroughly validated the methodology using various biological matrices and are set to use this technique now on the full set of cancer cell lines. With the increased throughput capacity of the mass spectrometry core, we do not anticipate any problems in making up for the initial delay.

Excitingly, through the new instrumentation we gained additional capabilities for quantitative comparisons of the peptide expression levels in cultured media. We successfully implemented a label-free, MS-1based area-under-the-curve quantitation method in addition to a targeted parallel reaction monitoring (PRM) approach. The MSl method is being used to quantify peptides in data-dependent acquisitions to create a baseline expression profile. The PRM method offers higher levels of sensitivity and specificity to target previously identified or known peptides. Finally, we also successfully implemented the WiSIM-DIA methodology that is unique to the Orbitrap Fusion instrument. WiSIM-DIA allows us to achieve quantitation at high-resolution (240,000) in a data-independent acquisition format – one of the latest trends in proteomics. The key advantage of this method is that in addition of offering higher levels of specificity, sensitivity and reproducibility, it acquires sequence information on all detected molecular species – which are available for re-interrogation downstream.

Due to the closing of Boston Biomedical Research Institute, we initially lost the peptide synthesis capabilities provided by our former in-house collaborator Dr. Paul Leavis. We were able to obtain the peptide synthesizer from BBRI and have now established anew an in-house peptide synthesis and purification workflow here at the Forsyth Institute. We have started synthesizing neuropeptides detected in prostate cancers that will be used downstream for the validation of peptide identification/quantitation methods and for neuroactivity measurements.

4. Key research accomplishments

- Successfully transferred existing workflows on the newly installed state-of-the-art instruments (Thermo Scientific Orbitrap Fusion; QExactive Plus) in the new mass spectrometry core facility at Forsyth.
- The new instrumentation with updated workflows now allows us to identify more peptides with higher accuracy and confidence.
- Established three complementary state-of-the-art mass spectrometry-based quantitation workflows that will be used for peptide quantitation: MSl-AUC; PRM and WiSIM-DIA.
- Established in-house peptide synthesis and purification workflow; synthesis of neuropeptides detected in prostate cancers

5. Conclusions

Despite the initial setback through the relocation/establishment of the new mass spectrometry core at the Forsyth Institute, we have made great strides toward accomplishing the goals of this grant. Specifically, we established improved methodologies that capitalize the full potential of the newly acquired, state-of-the-art instrumentation to obtain the highest quality of data currently achievable in terms of sensitivity, accuracy and reproducibility (qualitatively and quantitatively).

With the improved methodologies now firmly in place, we are in an ideal place to accomplish the remaining major tasks in the project.

6. Publications, abstracts and presentations
Nothing to report.

7. Inventions, patents and licenses
Nothing to report.

8. Reportable outcomes
Nothing to report.

9. Other achievements
Nothing to report.

10. References
Nothing to report.

11. Appendices

Appendix Al: Updated Facilities and Resource – Mass Spectrometry Core at the Forsyth Center for Salivary Diagnostics

Dr. Markus Hardt is the faculty director of the Mass Spectrometry Core of the Center, which currently houses a Thermo Scientific Orbitrap Fusion system coupled with a Dionex 3000 2D-nanoLC system, a Thermo Scientific QExactive Plus Orbitrap LC-MS/MS system coupled with a easy-nanoLC system, an AB Sciex 4800 Plus MALDI TOF/TOF system complemented by an AB Sciex Tempo LC MALDI Spotting system, a Protein Technologies Symphony automated 12 Channel solid phase peptide synthesizer along with a Dionex 3000 semi-preparative LC-system, and a BIAcore 3000 surface plasmon resonance instrument The Core is funded to acquire an additional LC-MS system dedicated to metabolomic workflows in budget year 2014/15. For data collection, processing and analysis, workstations with a wide range of specialized analyzed software packages are available as well as storage servers.